

“The artificial Insemination of Mammals and subsequent possible Fertilisation or Impregnation of their Ova.” By WALTER HEAPE,\* M.A., Trinity College, Cambridge. Communicated by FRANCIS GALTON, F.R.S. Received January 15,—Read February 11, 1897.

My attention was first drawn to this subject in consequence of the results obtained during an investigation, with which I have been occupied during the last three years, upon ovulation in the rabbit.

I found that “heat” alone was not sufficient to cause ovulation in the virgin rabbit, but that  $9\frac{1}{2}$  to 11 hours after copulation ovulation almost invariably took place. I then endeavoured to determine whether the act of copulation or the presence of spermatozoa in the female generative organs were independently sufficient to induce ovulation in does which were on “heat,” and I have made a considerable number of experiments for that purpose, an account of which I hope shortly to publish. Amongst them I made efforts to inseminate artificially virgin does when on “heat.” Seminal fluid was injected into the vagina of several of these animals, and in certain of them spermatozoa were subsequently found in the uterus and fallopian tubes. In no case, however, did the presence of spermatozoa thus artificially introduced induce ovulation, and it would seem that the presence of spermatozoa alone is not sufficient for the purpose.

Again, in a case where copulation took place, and where the os uteri of the doe was obstructed so as to stop the passage of spermatozoa into the uterus, ovulation did not take place, and thus it would seem that the act of copulation is not of itself sufficient for the purpose.

Such experiments as I have already made do not entirely satisfy me, but so far as they go they point to the conclusion that both copulation and the presence of spermatozoa in the uterus are necessary to induce ovulation in the virgin rabbit when she is on “heat.”

Ott (No. 24) experimented on sixteen rabbits, and claims to have induced gestation in one of them by artificially introducing spermatozoa through the body-wall into the peritoneal cavity near the ovary. His description is not very clear, but it would appear probable that he used for the experiment a doe rabbit which had kindled shortly before, and it is asserted by Weil (No. 39) that ovulation takes place spontaneously at such times in rabbits. This is a very interesting point, but I will not allude to it further here, as it properly concerns questions of ovulation and the maturation of ova which I propose to treat at length elsewhere.

\* With this is incorporated an account of experiments performed by Sir Everett Millais, Bart., and communicated by him to the author.

Certain other mammals, however, differ from the rabbit in the matter of ovulation. Sobotta (No. 34) has shown that mice ovulate prior to the act of copulation, and Tafani's (No. 36) researches point to the same conclusion for rats, though whether the animals used for these investigations were virgins or not is not stated.

Spallanzani (No. 35), Pierre Rossi (No. 27), and Sir Everett Millais's experiment on bitches, to which I refer below, show that the act of copulation is not necessary to bring about ovulation in these animals; certain other writers have shown that the same is true for the mare, and the researches of various medical experts demonstrate a similar possibility for the human female.

According to Hausmann (No. 12), sheep do not ovulate unless copulation takes place, but it is not improbable that many, if not most, of the lower mammals will be found to ovulate independently of the act of coition. In order to prove this fact, as my researches on the rabbit will show, other evidence must be sought for than the fallacious evidence of the appearance of the so-called corpora lutea in the ovaries (Pouchet, No. 26).

The possibility of artificially inseminating mammals in which ovulation takes place independently of coition, has seemed to me well worthy of notice, and the following short account has been written for the purpose of drawing attention to a subject which has apparently been much neglected, and with the hope that it may induce observers to record anew experiments of which I have been unable to find records and facts bearing upon it which are not already known. The importance of the subject is not, I think, fairly recognised, but I believe that a fuller knowledge of what is known about it will show that it is a matter of great interest both from a scientific and a practical point of view.

It has been ascertained that if spermatozoa be placed artificially in the vagina of certain female mammals at the right season, they may conceive; it has been ascertained also that if spermatozoa be placed artificially within the uterus of certain individual mammals which have failed for particular reasons to breed by natural means, they may become pregnant in consequence; and there is little doubt that an extended study of the subject will throw light on the physiological relations of coition and insemination, ovulation and fertilisation, and on certain of the causes which induce sterility in mammals, which will be of great interest to physiologists and of great value to practical breeders.

There is another, a wider but more problematical direction in which a fuller knowledge of the possibilities of artificial insemination may induce experiment; I mean the crossing of varieties or of species that will not naturally breed together.

I am not aware of any proof that any mammalian spermatozoon is

precluded from fertilising any mammalian ovum. The indiscriminate crossing of mammals is sufficiently guarded against naturally by the variation in size, in habit, and in the formation of the generative organs of the different species; it remains to be proved that the spermatozoa of one species will not fertilise the ova of other species and that the maternal organ will not receive and nourish the hybrid embryo.

I would here draw attention to the use of the word "inseminate." The terms "fertilisation" or "impregnation" have very generally, though as I believe erroneously, been used to denote the fact that the male generative substance has been introduced onto or into the generative organs of a female plant or animal, and it has even been assumed by some authors that the female herself has thus become "fertilised," or "impregnated." This assumption has probably given rise to some confusion, those who believe in telegony may, likely enough, be influenced by it. As far as is known, the female absorbs no essential part of the male elements with which she is supplied, although it may be found that she does so. However that may be, the female herself is not fertilised by means of pollen or spermatozoa, and her ova are not necessarily impregnated in consequence of the introduction into her generative organs of the male element. It has seemed necessary, therefore, to make a distinction between the introduction of seminal fluid into the female generative organs of animals and the subsequent possible fertilisation of their ova, and for that purpose I have used the word "inseminate," which can thus be applied to animals in precisely the same way as the word "pollenate" is applied by some botanists to denote the placing of pollen on the stigma of a plant.

Some time about the year 1770, Spallanzani (No. 35) made experiments on the artificial insemination of various oviparous animals and subsequently was induced to attempt a similar experiment on a mammal. He chose a bitch of the "Barbet" breed for the purpose and kept her in a room for a long time, feeding her himself.

She came "on heat" in due time and after the bleeding stage was over, showed strong desire for coition. Spallanzani then obtained from a young dog of the same breed, 19 grains of seminal fluid, and this, he says, he injected without delay into the "matrice" of the bitch by means of a small pointed syringe, taking care to keep the instrument the same temperature as the seminal fluid, viz., about 30° Réaumur. Two days after the injection the period of "heat" was finished; twenty days later the abdomen seemed swollen, and on the twenty-sixth day the bitch was set at liberty. The abdominal swelling increased, and sixty-two days after the injection was administered she gave birth to three pups.

On the 12th January, 1782 [according to Tarnier (No. 37), sixteen

months after Spallanzani had completed the experiment mentioned above], Pierre Rossi (No. 27) repeated this experiment. He also chose a "Barbet" bitch for the purpose. When she appeared to be coming "on heat" he locked her up in a room, to open which two keys were necessary; one of these he handed to M. Nicholas Branchi, Professor of Chemistry in the University of Pisa, and the other he held himself.

On the 25th January the bitch was ready for coition and spermatozoa was injected into her in the same way as Spallanzani describes his experiment. The injection was repeated on the 26th, 28th, and 30th of the same month. On the 1st February "heat" ceased, on the 26th the abdomen was swollen and the mammæ enlarged, and the bitch was set at liberty. On the 27th March, sixty-two days after the first injection, she littered four young.

The care with which both, but especially the latter, of these experiments was conducted, leaves little room for doubt that the bitches produced young in consequence of artificial insemination.

It is a matter for surprise that such extremely interesting experiments did not stimulate research in the same direction. They do not, however, appear to have done so, at any rate I can find no reference since Spallanzani's day until quite recently, of any experiments on the artificial insemination of the lower mammals.

The human female, on the other hand, has been made the subject of like experiments by various medical men. Hunter (No. 14), in 1799, seems to have been the first to advise the artificial insemination of woman in order to insure pregnancy. In the case which he records, the operation was performed in consequence of the fact that the husband of the woman suffered from hypospadias, and the seminal fluid was merely injected into her vagina. It is recorded that success attended the operation and that the woman became pregnant and bore a child.

Since Hunter's day various medical men have practised artificial insemination on women who were supposed to be sterile, with more or less success. Their method has been, to inject spermatozoa into the uterus of the woman by means of a specially devised instrument and in that way to overcome certain defects or obstructions in the female generative organs; such as flexion or constriction of the canal of the cervix; rigidity of the cervix; hypertrophy of the lips of the external os which may effectually close the orifice; inability to retain the spermatozoa in the vagina owing to violent muscular contraction, or to retroversion of the uterus (Sims, No. 33); a want of sufficient muscular power; abnormal structure or size of the cervix or os uteri which prevents the free action of the functions of the cervix ["aspirations," Courty (No. 3)]; abnormal or excessive vaginal secretions, &c.

Such defects as these may effectually prevent conception by natural means, since spermatozoa placed in the vagina may thus be prevented from ascending to the uterus and fallopian tubes and from meeting there a newly discharged ovum, but they may be overcome by placing the spermatozoa directly into the uterine cavity.

The great difficulty of ensuring success with women would seem to be due to our ignorance of the time of ovulation. I will not refer to this matter further here, having considered it somewhat fully elsewhere (No. 13).

The performance of the operation on women presents no great difficulties and writers on the subject are agreed on this point. Spermatozoa is collected, either direct from the male, or from the vagina of the female immediately after coition, and is injected into the cavity of the uterus by means of a syringe, to which is attached a fine tube which the operator passes through the canal of the cervix.

Tarnier (No. 37) credits Girault with having operated on forty women who were positively declared to be sterile, of which number eighteen bore children in consequence, and his seems to have been the most successful record. I have given at the end of this paper a list of the authors who deal with the subject, and it will not be necessary to go more fully into this branch of it here, since the writers record nothing which is of further general interest.

In recent years both bitches and mares have been successfully inseminated by artificial means.

Sir Everett Millais, who is a noted breeder of Basset hounds, has made a very remarkable series of experiments of this kind on bitches in his own kennels, and he has most kindly supplied me with an account of them and placed the details at my disposal for publication. His method is to collect spermatozoa from the dog and inject it by means of a syringe into the generative canal of the bitch; it seems probable that the syringe does not reach beyond the vagina and that the spermatozoa is thus deposited at the further end of that organ, from whence it finds its way into the uterus by the same means employed after natural insemination. These experiments were not undertaken in order to overcome barrenness; hence it was quite sufficient for his purpose to inject the spermatozoa into the vagina.

*Experiment 1.*—In 1884 Sir Everett Millais published (No. 19) an account of the first experiment he made. He inseminated artificially a Basset hound bitch with spermatozoa obtained from a dog of the same breed, with the result that the bitch gave birth to pups, which were, however, born dead.

*Experiment 2.*—In 1885 he artificially inseminated three Basset hound bitches, the semen being obtained from a single emission of a dog of the same breed, and each bitch receiving one-third part of it.

All these bitches pupped successfully, one having a single puppy, another five puppies, and the third seven puppies.

*Experiment 3* (1887) was a failure; the bitch used for this experiment invariably refused to allow a dog to serve her when "on heat," and although she offered no objections to the performance of artificial insemination she failed to conceive.

*Experiment 4* (1891). A Bloodhound bitch was artificially inseminated with material obtained from the Basset hound dog "Floreal;" a single puppy was the result, and it resembled anatomically its sire.

*Experiment 5* (1891) was not satisfactory, a miscarriage taking place.

*Experiment 6* (1891) was a failure; the bitch was a noted bad breeder and failed to conceive.

*Experiment 7* (1891) was a failure; the dog used was a very old one and it is possible the failure was due to him.

*Experiment 8* (1891) was also a failure, and in this case also the bitch used was a bad breeder, having only produced one puppy in her life.

*Experiment 9* (1892). The Basset hound bitch "Antoinette" was artificially inseminated with semen from the dog "Floreal," and a litter of seven puppies was the result, one of them, "Syringa," being as he says, "the best puppy I ever bred."

*Experiment 10* (1893). A Bloodhound bitch was artificially inseminated with semen taken from a Basset hound and immediately afterwards she was served naturally with a dog of her own breed, "Dandy." This bitch littered eleven puppies, eight of which were pure bred Bloodhounds, and three were half-bred Basset and Bloodhounds.

*Experiment 11* (1893). The Basset hound bitch "Bud" was artificially inseminated with semen from the dog "Floreal," six puppies resulting.

*Experiment 12* (1893). A Bloodhound bitch was inseminated artificially with semen from the Basset hound "Nicholas." She had twelve puppies, all resembling their sire anatomically. As this bitch was dying in labour, these puppies were delivered by Cæsarian section, and seven of them were reared.

*Experiment 13* (1894). A Bloodhound bitch, "Garnet," was artificially inseminated with semen from the Basset hound "Floreal." One bitch puppy, "Bella," was born; she resembled anatomically her sire, and she has since been bred from.

*Experiment 14* (1894). A Basset hound bitch, "Juno IV," was artificially inseminated with semen from a Basset hound dog champion, "Forrester," and eight puppies were born in consequence.

*Experiment 15* (1894). One of the half-bred bitches obtained by Experiment 12 was artificially inseminated with semen from the dog "Floreal," and she littered nine puppies.

*Experiment 16.*—A three-quarter bred bitch, "Betsy," whose dam was one of the puppies resulting from Experiment 12, was artificially inseminated with semen obtained from the Basset hound dog champion "Forrester," and nine puppies were born.

*Experiment 17* (1896). The artificial insemination of another Basset hound bitch has resulted in the birth of puppies.

Nineteen bitches were artificially inseminated in these seventeen experiments, and only four of them failed to conceive. Of these four, one bitch invariably refused the dog, two bitches were known to be uncertain breeders, and the fourth was injected with semen obtained from an old dog and it is possible the failure to breed in this case may be due to the dog and not to the bitch.

This remarkable series of experiments, extending over twelve years, is of very great interest. All possible care was taken by the owner, an experienced breeder, whose kennels are admirably organised, that no error should creep in, and it may be confidently stated as a result of them—

First, that artificial insemination of bitches can readily be performed; secondly, that pregnancy can be as readily induced by that means as by the natural method of coition; and, thirdly, that a single emission of spermatozoa by a dog can be made use of to inseminate artificially several bitches, all of which may be expected to bear pups.

Another interesting fact which is demonstrated by these experiments is the facility artificial insemination affords of obtaining crosses between animals which, on account of the great difference in their size (as in Basset and Bloodhounds) cannot readily be obtained by natural means; while a further matter of interest, of no little importance to students of heredity and telegony, is the demonstration of the possibility of inseminating a single bitch with the semen of two or more dogs of different breeds at the same time, and of inducing her by this means to produce a variety of crosses in a single litter.

Gautier (No. 7) also claims to have artificially inseminated a bitch, but he gives no details of the operation, and I have been unable to find records of any other experiments of this nature on dogs.

With regard to horses, Gautier (No. 7) cites Dr. Le Bon as responsible for a quotation from a book written in the year 700 of the Hejira, from which it appears possible that artificial insemination of mares was not unknown to the Arabs in those days.

He describes how the owner of a valuable mare "on heat," armed with a handful of cotton-wool which had been saturated with the discharge from the vagina of the mare, approached by stealth a valuable stallion belonging to a member of a neighbouring hostile

tribe, and, having sufficiently excited the animal with the scent of the material he had brought, collected from him spermatozoa, which he introduced on his return into the vagina of his mare, and obtained thereby a foal.

The story as it stands is sufficiently improbable, but it would seem almost as improbable that such an incident as is here described could have been invented or imagined in those early days, and, in view of the fact that artificial insemination of mares is actually practised now, though in a very different manner, the improbability of this story is, to some extent, decreased.\*

The only modern evidence I have been able to obtain regarding the artificial insemination of mares, comes from the United States.

A writer on "Breeding Mares," in 'The Horseman' (No. 1), 1894, speaks of the artificial insemination of mares as of established usefulness, giving positive results.

He gives as an example his experience on a farm where the stallion was of "faulty formation," and the mares were not "settling." At the time of his visit four mares were in season; by his advice one of them was served by the stallion, and the other three were artificially inseminated with semen taken from the vagina of this first mare.

The result of the experiment was that the mare which was actually served by the stallion failed to become pregnant, while the three which were artificially inseminated were all got with foal. Subsequently, the writer adds, twenty-five mares on this same farm, which had previously missed getting with foal in the ordinary way, were operated on, and twenty-three of them became pregnant in consequence.

A writer on "Artificial Impregnation," in the same paper (No. 2), 1895, quotes a letter from a breeder in Harrison, Ill., dated August, 1894, from which it appears that the previous season this gentleman had seventeen mares which had refused to "stand to the horse." All of them had refused for two years past, some for several years; these mares were artificially inseminated and nine foals were in consequence produced.

In this article it is recorded that one mare, sixteen years old, which had repeatedly failed to produce young in the ordinary way, was operated on in July, 1893, and bore a filly in June, 1894; while a jennet, twenty-five years old, which had not bred for six years, foaled a colt in June, 1894, after artificial insemination in June of the previous year.

There can be little doubt of the *bona fides* of these reports, but in

\* Note, February 11.—In connection with this story I should have added here, that the spermatozoa of a dog was sent to me by post, during the early spring of 1896, and, when examined eighteen hours after it was obtained from the dog, I found fully half of the spermatozoa active and vigorous.



order to confirm them, Professor Pearson, Veterinary Professor at the University of Pennsylvania, Philadelphia, was applied to, and Mr. Francis Galton very kindly forwarded to me a letter from Professor Pearson, in which he states that the artificial insemination of mares is practised on a number of stock-farms in America with fairly satisfactory results; and further, he adds, he himself has performed the operation and attained success.

The usual method adopted is, to allow the stallion to cover a mare in the ordinary way, then, immediately after coition, to pass a hand into the vagina carrying therein the nozzle of a specially constructed syringe, to depress with the tips of the fingers the ventral wall of the vagina at its anterior end, and draw up into the syringe the seminal fluid which will collect in the depression so formed. The same mare can then be inseminated by inserting the nozzle of the syringe through the external os uteri and forcing some of the semen into the uterine cavity, then the syringe can be withdrawn, and other mares which are "in use" can be inseminated with the remainder of the semen in a similar way.

Another method is also in use in the United States, and in this case gelatine capsules are used instead of a syringe; these capsules are filled with spermatozoa from the vagina of a mare which has been covered; they are then closed and gently forced through the external os into the uterus and allowed to remain there; the heat of the body melts the gelatine, and the semen is set free.

It is possible by either of these methods to inseminate several mares with semen obtained from one single act of coition, and in this way the usefulness of a valuable stallion may be vastly increased.

The above operations on mares were conducted for the purpose of overcoming certain causes which induced sterility, and for this reason the semen was introduced directly into the uterine cavity. What these causes actually were is not stated, but it is obvious that certain defects which prevent mares from breeding in the ordinary way can be overcome by artificially introducing the spermatozoa of a horse into their uterus.

The causes of sterility in mares are, apparently, very various. Fleming (No. 6), in the couple of pages which he devotes to the subject, briefly refers to many of them. Here we are only concerned with the causes which prevent the passage of live spermatozoa from the vagina into the uterus of an otherwise normally constituted animal.

An abnormal condition of the cervix uteri is more common than is generally known, according to Fleming; occlusion of the os, due to a spasmodic contraction of the muscles of the cervix during coition; rigidity of the cervix; hypertrophy of the tissue of the os; and the formation of false membranes, which serve to close the entrance to

the uterus; are all causes which may be overcome by the method of artificial insemination described above.

Then, again, abnormal vaginal secretions may kill the spermatozoa; and another not uncommon cause of sterility in the mare, not mentioned by Fleming, is the inability of the vagina to retain the semen after coition; in such cases the withdrawal of the horse is immediately followed by ejection of the semen from the vagina. This fault may be due either to violent muscular contraction of the vagina, or to an abnormally short vagina which is stretched by the penis during coition, and which springs back again rapidly when that organ is withdrawn. Artificial insemination is readily applicable also in the latter case; the semen is collected in a basin as it falls from the vagina, is rapidly drawn up into a syringe, and injected into the uterus in the way described above.

With regard to the number of cases of sterility in mares, there is no doubt that the percentage varies in different years, and that some seasons appear to be more favourable for breeding than other seasons. According to the evidence obtained by the Royal Commission on Horse Breeding (Nos. 29, 30, 31, 32) it is probably correct to estimate that about 40 per cent. of breeding mares which are put to the horse fail to produce offspring each year.

This is a very considerable proportion, and it would appear highly probable that breeders of valuable animals would gladly adopt a method of reducing the loss occasioned by the infertility of so large a proportion of mares, provided they could be assured that harm cannot be done to breeding stock by the process, and provided also that the offspring so obtained should be as healthy and vigorous as that got in the ordinary way.

The operation itself is perfectly simple, and cannot possibly do harm if conducted by a qualified person; it causes neither pain nor inconvenience to the mare.

With regard to the offspring begot by this process, I have no evidence bearing on their vigour or health, and no doubt it is important to the breeder to know this. The best way of solving the question would be to use thoroughbred mares for the experiment, and to subject the foals so obtained to the same rigorous tests usually accorded to such animals; their health, vigour, and stamina would by this means be readily and justly judged.

By the kindness of Lord Rosebery I was enabled late last spring to superintend the artificial insemination of the thorough-bred mare "Myra." This mare has proved an uncertain breeder, and during the whole of last breeding season she had failed to stand to the horse. She was covered in my presence, and the seminal fluid drawn up from her vagina into a syringe; some slight difficulty was at first experienced in passing the nozzle of the syringe through the os,

owing probably to hypertrophy of the lips of the os, but the difficulty was readily overcome without annoyance to the mare and she was inseminated. Unfortunately the experiment failed and she has not proved to be with foal.

Whether the failure in this case was due to abnormal vaginal secretion, with which, of course, the semen was mixed when injected into the uterus, or to the absence of ova, or to faulty powers of gestation, I have no means of knowing. Many experiments are needful in order to enable one to form a satisfactory opinion on such points; but there is good reason to believe that, with experience, they may be determined, and it is greatly to be hoped that breeders will be found who are willing to allow such of their stock as are unsatisfactory breeding animals, to be treated in this way before finally discarding them as useless.

Cows are frequently sterile for reasons similar to these detailed for the mare, hypertrophy of the mucous membrane of the os uteri and consequent closure of the cervix canal being a common cause of sterility in these animals.

It is said that artificial insemination can be performed on cows as readily as it can on mares, but I have seen no published account of experiments on cows.

---

*Literature.*

- No. 1. A writer. "On breeding mares," 'The Horseman.' Chicago, March 8, 1894.
- „ 2. A writer. "Artificial Impregnation," 'The Horseman.' Chicago, May 30, 1895.
- „ 3. Courty. "Traité pratique des maladies de l'utérus des ovaires et des trompes."
- „ 4. Dehaut. "De la fécondation artificielle dans l'espèce humaine comme moyen de remédier à certaines causes de stérilité chez l'homme et chez la femme." Paris, 1865.
- „ 5. Eustache (referred to by Leblond, No. 14).
- „ 6. Fleming. "A Text-book of Veterinary Obstetrics," 1878.
- „ 7. Gautier. "La fécondation artificielle et son emploi contre la stérilité chez la femme." Paris, 1889.
- „ 8. Gigon. "Essai sur la fécondation artificielle de la femme." Thèse de doctorat. Paris, 1871.
- „ 9. Gigon (d'Angoulême). "Réforme Médicale," Sept. 29, 1867.
- „ 10. Girault. "Etudes sur la génération artificielle dans l'espèce humaine," 'l'Abeille Médicale,' Nov. 30, 1868.
- „ 11. Godard. "Égypte et Palestine, Observations médicales et scientifiques." Paris, 1867.
- „ 12. Hausmann. "Ueber die Zeugung und Entstehung des wahren weiblichen Eies bei den Säugethieren und Menschen." Hanover, 1840.
- „ 13. Heape. "The Menstruation of *Semnopithecus entellus*," 'Philosophical Transactions of the Royal Society,' B, vol. 185, 1894.

- No. 14. Hunter. Home's "Account of the Dissection of an Hermaphrodite Dog," 'Philosophical Transactions of the Royal Society,' p. 162. 1799.
- „ 15. Leblond. "Traité élémentaire de chirurgie gynécologique." Paris, 1878.
- „ 16. Leblond. "Note sur la fécondation artificielle à propos d'un jugement du tribunal de Bordeaux," 'Annales de Gynécologie,' vol. 20. Paris, 1883.
- „ 17. Leseur. 'La Réforme Médical.' Paris, 1867.
- „ 18. Lutard. "A la fécondation artificielle," 'Courier Médical,' vol. 29, Paris, 1879.
- „ 19. Millais. "An Artificial Impregnation," by a Dog Breeder. 'Veterinary Journal,' vol. 18, 1884.
- „ 20. Millais. "Influence with Special Reference to that of Sire," 'Dog Owner's Annual,' 1894.
- „ 21. Millais. "Two Problems of Reproduction," Our Dogs' Publishing Company, 1895.
- „ 22. Manant Taberner. "La fecundacion artificial," 'Encycl. Med. farm.' Barcelona, 1880.
- „ 23. Manant Taberner. "Fecundacion artificial, historia, indicacion y procedes," 'Encycl. Med. farm.' Barcelona, 1884.
- „ 24. Ott. "Ueber künstliche Befruchtung durch die Peritonealhöhle," 'Centralblatt für Gynaekologie,' No. 36, 1882.
- „ 25. Pajot. "Examen général du traité du Dr. Courty sur les affections utérines," 'Archives Général de Médecine,' vol. 9.
- „ 26. Pouchet. "Théorie positive de l'ovulation spontanée." Paris, 1847.
- „ 27. Rossi. "Fécondation artificielle, opérée sur un chien," Tirée des 'Opuscoli Scelti' di Milan, vol. 5 (see Spallanzani, No. 31).
- „ 28. Rouband. Académie de Médecine, April 12, 1872. See also article "Génération," 'Nouveau Dictionnaire de Médecine et de Chirurgie pratiques,' vol. 15. See also "Traité de l'impuissance et de la stérilité chez l'homme et chez la femme," 3rd Ed. Paris, 1876.
- „ 29. Royal Commission on Horse Breeding, 1st Report, 1888.
- „ 30. " " " 2nd Report, 1888.
- „ 31. " " " 3rd Report, 1890.
- „ 32. " " " 4th Report, 1893.
- „ 33. Sims. "Clinical Notes on Uterine Surgery," 1866.
- „ 34. Sobotta. "Die Befruchtung und Furchung des Eies der Maus," 'Archiv für Mikroskop. Anatomie,' vol. 45, 1895.
- „ 35. Spallanzani, "Expériences pour servir à l'histoire de la génération des animaux et des plantes." Genève, 1786.
- „ 36. Tafani. "La fécondation et la segmentation étudiées dans les œufs des rats," 'Archives Italiennes de Biologie,' vol. 2, 1889.
- „ 37. Tarnier. "Traité de l'Art des Accouchements." Paris, 1878.
- „ 38. Vulliet et Lutard. "Leçons de Gynécologie opératoire." 10th Edit. Paris, 1890.
- „ 39. Weil. "Beiträge zur Kenntniss der Befruchtung und Entwicklung des Kanincheneies," 'Wien. Mediz. Jahrbuch,' 1873.